

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 5 :</b> <b>C12Q 1/68, C07H 21/04</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 90/08841</b> <b>(43) International Publication Date:</b> 9 August 1990 (09.08.90)
<b>(21) International Application Number:</b> PCT/US90/00656 <b>(22) International Filing Date:</b> 5 February 1990 (05.02.90) <b>(30) Priority data:</b> 307,023 6 February 1989 (06.02.89) US <b>(71) Applicant:</b> GENE-TRAK SYSTEMS [US/US]; 32 New York Avenue, Framingham, MA 01701 (US). <b>(72) Inventors:</b> KING, Walter ; 3 Fletcher Street, Maynard, MA 01754 (US). SHAH, Jyotsna, S. ; 13 Bates Drive, Nashua, NH 03060 (US). NIETUPSKI, Raymond, M. ; 6 Cherry Street, Millbury, MA 01527 (US). RAPOSA, Susan ; 117 Spring Street, Cambridge, MA 02141 (US). WARSHAW, Jane ; 15 Capital Street, Newton, MA 02158 (US). GROODY, Patrick ; 249 Wilson Street, Marlboro, MA 01752 (US). LAWRIE, Jonathan ; 5 Oak Terrace, Milford, MA 01757 (US). PARSONS, George ; 23 Brewster Road, Arlington, MA 02174 (US). LANE, David, J. ; 9 Oriole Drive, Milford, MA 01757 (US).		<b>(74) Agent:</b> HOFER, Mark, A.; Genzyme Corporation, One Mountain Road, Framingham, MA 01701 (US). <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PROBES AND METHODS FOR THE DETECTION OF LISTERIA  <b>(57) Abstract</b>  Improved nucleic acid probes capable of specifically hybridizing to rRNA of <i>Listeria</i> and not to rRNA of non- <i>Listeria</i> are described along with methods utilizing such probes for the detection of <i>Listeria</i> in food and other samples.		

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic	KR	Republic of Korea	SN	Senegal
CG	Congo	LI	Liechtenstein	SU	Soviet Union
CH	Switzerland	LK	Sri Lanka	TD	Chad
CM	Cameroon	LU	Luxembourg	TG	Togo
DE	Germany, Federal Republic of	MC	Monaco	US	United States of America
DK	Denmark				

Probes and methods for the detection of Listeria.

5

Field of the Invention

10

This invention relates to detecting bacteria belonging to the genus Listeria and more specifically provides improved nucleic acid probes and compositions along with methods for their use for the specific detection of Listeria.

Background of the Invention

15

The term "Listeria" as used herein, refers to the bacteria classified as such in Bergey's Manual of Systematic Bacteriology (P. H. A. Sneath (ed), 1986, 1234-1245, Williams & Wilkins). Recent 16S rRNA sequence data of Listeria murrayi and Listeria grayi together with well known phenotypic uniformities have led to a recent proposal to retain these two groups in the Listeria genus (Rocourt et al., International Journal of Systematic Bacteriology, Vol. 37, No. 3, pp 298-300 (1987)). Therefore, the term "Listeria" as used herein includes Listeria monocytogenes, Listeria innocua, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii, Listeria murrayi and Listeria grayi.

25

Detection of Listeria is important in various medical and public health contexts. Human listeriosis has been shown to be clearly related to consumption of Listeria-contaminated foods only in the past few years. The outbreaks documenting this association (Schlech et al., New England J. Med. 308:203-206 (1983); Fleming et al., New Eng. J. Med. 312:404-407 (1985); U.S. Public Health Service, Centers for Disease Control, Morb. Mortal. Rpt. 34:357-359 (1985)) have caused concern among public health officials, food manufacturers and regulatory agencies. Prior to these outbreaks, food microbiologists have been generally unaware and uninformed about this nearly

30

35

ubiquitous group of organisms and the associated disease manifestations. Most efforts to improve diagnostic methods for Listeria have been based on its occurrence as a veterinary pathogen or as a cause of human neonatal infection (Hird, J. Food Protection 50:429-433 (1987)).

Many samples cultured for Listeria (including foods) contain large, mixed microbial populations which hamper recovery and identification. Therefore, it is an aspect of the present invention to provide a novel assay system capable of rapidly detecting Listeria and which is generally applicable to environmental, food or clinical samples.

Because Listeria species other than L. monocytogenes have been recovered from a variety of food types, detection of such organisms in a food-processing environment may be important by virtue of their "indicator organism" status.

The presence of Listeria has been historically detected by culturing an appropriately prepared sample on microbiological media under conditions favorable for growth of these organisms (Lovett et al., J. Food Protection 50:188-192 [1987], Anonymous, Fed. Register 53: 44148-44153 [1988], McClain and Lee, J. Assoc. Off. Anal. Chem., 71:660-664 [1988]). The resulting colonies are typically examined for morphological and biochemical characteristics, a process that generally is initiated 48 hours after acquisition of the sample and disadvantageously takes between 4-5 days to complete.

It is another aspect of the present invention to avoid the disadvantage associated with traditional, multi-day culturing techniques and to employ nucleic acid probes to detect Listeria.

It is yet another aspect of the present invention to provide probes which can hybridize to target regions which can be rendered accessible to the probes under normal assay conditions.

While Kohne et al., Biophysical Journal 8:1104-1118 (1968), discuss one method for preparing probes to rRNA sequences they do not provide the teaching necessary to make Listeria specific probes.

- 5        Copending USSN 096,570 of Stackebrandt et al., filed 9/11/87 describes Listeria specific probes and while such probes work well, it is another aspect of the present invention to provide novel and improved Listeria probes not disclosed therein.
- 10        Pace and Campbell, Journal of Bacteriology 107:543-547 (1971), discuss the homology of ribosomal ribonucleic acids from diverse bacterial species and a hybridization method for quantitating such homology levels. Similarly, Sogin, Sogin, and Woese, Journal of Molecular Evolution 1:173-184 (1972), discuss the theoretical and practical
- 15        aspects of using primary structural characterization of different ribosomal RNA molecules for evaluating phylogenetic relationships. Fox, Pechman, and Woese, International Journal of Systematic Bacteriology (1977), discuss the comparative cataloging of 16S ribosomal RNAs as an approach to prokaryotic systematics. These
- 20        references, however, fail to relieve the deficiency of Kohne's teaching with respect to Listeria and in particular, do not provide Listeria specific probes useful in assays for detecting Listeria in food and other samples.
- 25        Ribosomes are of profound importance to all organisms because they serve as the only known means of translating genetic information into cellular proteins, the main structural and catalytic elements of life. A clear manifestation of this importance is the observation that all cells have ribosomes.
- 30        Ribosomes contain three distinct RNA molecules which, at least in E. coli, are referred to as 5S, 16S, and 23S rRNAs. These names historically are related to the size of the RNA molecules, as determined by their sedimentation rate. In actuality, however,
- 35        ribosome molecules vary substantially in size between organisms.

Nonetheless, 5S, 16S, and 23S rRNA are commonly used as generic names for the homologous RNA molecules in any bacteria, and this convention will be continued herein.

5 Hybridization traditionally is understood as the process by which, under predetermined reaction conditions, two partially or completely complementary single-stranded nucleic acids are allowed to come together in an antiparallel fashion to form a double-stranded nucleic acid with specific and stable hydrogen bonds.

10

The stringency of a particular set of hybridization conditions is defined by the base composition of the probe/target duplex, as well as by the level and geometry of mispairing between the two nucleic acids.

15

Stringency may also be governed by such reaction parameters as the concentration and type of ionic species present in the hybridization solution, the types and concentrations of denaturing agents present, and/or the temperature of hybridization. Generally, as hybridization conditions become more stringent, longer probes are preferred if stable hybrids are to be formed. As a corollary, the stringency of the conditions under which a hybridization is to take place (e.g., based on the type of assay to be performed) will largely dictate the preferred probes to be employed. Such relationships are well understood and can be readily manipulated by those skilled in the art.

20

As a general matter, dependent upon probe length, such persons understand stringent conditions to mean approximately 35°C-65°C in a salt solution of approximately 0.9 molar.

25

As used herein, probe(s) refer to synthetic or biologically produced nucleic acids (DNA or RNA) which, by design or selection, contain specific nucleotide sequences that allow them to hybridize under defined predetermined stringencies, specifically (i.e., preferentially) to target nucleic acid sequences. Two functionally modified probes are described in the examples described herein.

30

35

Capture probes are modified in such a way that they hybridize to the target nucleic acid molecule and can be removed, along with the target molecule, from solution. Detection probes are advantageously modified by the addition of one of a variety of detectable ligands (e.g. <sup>32</sup>P, biotin, fluorescein, etc.) which permit the direct or indirect detection of the target nucleic acid.

A target nucleic acid sequence is one to which a particular probe is capable of preferentially hybridizing.

Still other useful definitions are given as their first use arises in the following text. All references cited herein are fully incorporated by reference.

#### Summary of the Invention

In accordance with the various principles and aspects of the present invention, there are provided improved nucleic acid probes and probe sets comprising DNA or RNA sequences which, under specific hybridization conditions, are capable of detecting the presence of ribosomal RNA (rRNA) molecules of Listeria or the DNA encoding such ribosomal molecules (rDNA), but which are not capable, under the same conditions, of detecting the rRNA or rDNA of other related bacteria which may be present in the test sample.

The present invention also features an assay system for the advantageous utilization of these probes, the format of which can enhance the aforementioned desirable behavior of the probes. Ideally, a cultivation step which enhances the growth of Listeria relative to that of other bacteria is included in the most preferred embodiment of the present assay. The microbiological selection obtained with such a cultivation step, in combination with the specificity of the

described probes, has been discovered to impart enhanced performance capabilities with respect to other currently available means for detection of Listeria generally including:

- 5           a) increased sensitivity; i.e., the ability to detect Listeria in a given sample more frequently than currently available methods;
- 10           b) potentially significant reductions in assay cost due to the use of inexpensive reagents and reduced labor;
- c) accurate identification of Listeria; and
- 15           d) faster results because the test does not require the isolation of Listeria from the cultured samples prior to testing. Accordingly, in the most preferred embodiment of the assay, only two days are required to provide a result.

20           The probes of the present invention impart enhanced performance with respect to those described in copending USSN 096,570 of Stackebrandt et al. in that they do not react with Brochothrix, a genus closely related to Listeria by genetic and biochemical criteria. Brochothrix is known to occur in some food and environmental samples which also support the growth of Listeria. Therefore, this characteristic of the present

25           probe set represents a significant enhancement in performance.

            It has been discovered that other advantages incurred by directing the probes of the present invention against rRNA include the fact that the rRNAs detected constitute a significant component of cellular mass.

30           Although estimates of cellular ribosome content vary, actively growing Listeria bacteria may contain upwards of  $1.0 \times 10^4$  ribosomes per cell, and therefore  $1.0 \times 10^4$  copies of each of the rRNAs (present in a 1:1:1 stoichiometry in ribosomes). In contrast, other potential cellular target molecules such as genes or RNA transcripts thereof, are

35           less ideal since they are present in much lower abundance.



- A further unexpected advantage is that the rRNAs (and the genes encoding them) appear not to be subject to lateral transfer between contemporary organisms. Thus, the rRNA primary structure provides an organism-specific molecular target, rather than a gene-specific target as would likely be the case, for example of a plasmid-borne gene or product thereof which may be subject to lateral transmission between contemporary organisms.
- 5
- 10 Additionally, the present invention provides probes to Listeria rRNA target sequences which appear to be fully inclusive for all Listeria strains. A most preferred embodiment comprises a mixture of three probes for hybridization to the rRNA in all Listeria.
- 15 Most of the specificity of the aforementioned mixture resides in one of the probes, the capture probe. Advantageously, the rRNA target sequence of the capture probe is sufficiently different in most non-Listeria rRNAs that, under the preferred assay conditions of the present invention, the capture probe of the present invention
- 20 hybridizes to Listeria rRNAs and does not hybridize with non-Listeria organisms. These probe characteristics are defined as inclusivity and exclusivity, respectively, and under the preferred assay conditions of the present invention are imparted to the aforementioned probe mixture.
- 25 The discovery that probes could be generated with the extraordinary and improved inclusivity and exclusivity characteristics of those of the present invention with respect to Listeria was unpredictable and unexpected.
- 30 In a preferred embodiment of the invention, an assay method for detecting Listeria is provided in which bacteria in the sample to be tested are preferably grown for a limited time under conditions which foster rapid and abundant growth of any Listeria in the sample and

which are biased against the growth of many closely related bacteria. Hybridization analysis using the preferred probes of the present invention is then advantageously performed on the sample after this growth period.

5

#### Brief Description of the Tables

Further understanding of the principles and aspects of the present invention may be made by reference to the tables wherein:

10

Figure 1: Shows alignment of the nucleotide sequences of the preferred probes of the present invention with the nucleotide target sequence including the "core" region from 456 to 477 of Listeria 16S rRNA (using the E. coli position numbering convention) along with relevant portions of the 16S rRNAs from Listeria monocytogenes, Listeria innocua, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii, Listeria grayi, Listeria murrayi and Brochothrix thermosphactata, Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Streptococcus epidermidis, Enterococcus faecalis, Clostridium innocuum, Mycoplasma capricolum, Arthrobacter globiformis and Streptomyces violaceoruber. RNA sequences are written 5' to 3', probe sequences are DNA and written 3' to 5'. Lower case c in certain of the probes indicates a modified cytosine residue to which a reporter group may or may not be attached depending on the assay form employed.

15

20

25

30

Probes of the 1120 series (1120, 1150, 1151 and 1152) are shown, along with the "core" region of variation upon which they are based, above the "parent" sequence

for Listeria monocytogenes. Much of the specificity of the preferred assay of the present invention resides in this series of probes, in which they are used as "capture" probes.

5

Probes 1118, 119 and 1212 are detection probes which are designed to be used with the 1120 series capture probes.

- 10      Table 1:      Exemplifies the inclusivity behavior of the preferred probe set toward a representative sampling of Listeria strains. Inclusivity data were obtained from the preferred liquid hybridization testing format of Example 1 (General).
- 15      Table 2:      Exemplifies the exclusivity behavior of the preferred probe set toward a representative sampling of non-Listeria strains. Exclusivity data were obtained from the preferred liquid hybridization testing format of Example 1 (General).
- 20      Table 3:      Provides data showing detection of Listeria in food samples with the preferred probes in the preferred format of the present invention (Example 1, Specific).

25                      Detailed Description of the Invention and Best Mode

Probe Development Strategy

30      The first step taken in the development of the probes of the present invention involved identification of regions of 16S and/or 23S rRNA which potentially could serve as target sites for Listeria specific nucleic acid probes. As a practical matter, it is difficult to predict, a priori, which non-Listeria organisms might be present in any test sample.

35

Because of the large number of such potential non-Listeria bacteria, demonstrating exclusivity for any given probe sequence is not only unpredictable but also extremely difficult and laborious. A more rigorous criterion was adopted to obviate the need to know what non-Listeria bacteria might be present in all test samples that ultimately will be screened using the probes.

This entailed knowledge of the phylogenetic relationships among Listeria and between Listeria and other groups of bacteria.

Specifically, an operating but previously unproven hypothesis was adopted that the exclusivity criterion could be satisfied by determining that if a particular target region in Listeria rRNA could be identified which was sufficiently different from the homologous region in the rRNA of representative yet close evolutionary relatives of Listeria, then a probe to such a sequence could be used to distinguish between Listeria and the relatives by hybridization assay. Based on phylogenetic observations, it then was extrapolated that rRNA sequences of more distantly related organisms, even though their actual identity may not necessarily be known, should be predictably different in a particular region of sequence than the aforementioned close evolutionary relative of Listeria. However, it cannot be predicted, a priori whether such regions exist or if they do, where within the rRNA such regions will be located.

As the first step in identifying regions of Listeria rRNA which could potentially serve as useful target sites for nucleic acid hybridization probes, nearly complete nucleotide sequences of the 16S rRNAs from isolates representing all seven Listeria species were determined including five isolates of L. monocytogenes and two isolate of L. innocua. These were arbitrarily selected as representative of the evolutionary breadth of genus Listeria.

The nucleotide sequences of various portions of the rRNAs were determined by standard laboratory protocols either by cloning (Maniatis

et al., 1982, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, pp 545) and sequencing (Maxam & Gilbert, 1977, Proceedings of the National Academy of Science, USA 74:560-564; Sanger et al., 1977, Proceedings of the National Academy of Science, USA 74:5463-5467) the genes which specify the rRNAs, and/or by direct sequencing of the rRNAs themselves using reverse transcriptase (Lane et al., [1985], Proceedings of the National Academy of Science, USA 82:6955-6959).

10 The determined Listeria rRNA nucleotide sequences were compared to one another and to other available rRNA nucleotide sequences, in particular to those of closely related bacteria such as Brochothrix, Staphylococcus, Streptococcus, Enterococcus, and Bacillus, which also were determined as part of this work.

15 One region of sequence was identified which appeared to be conserved among all Listeria and which, importantly, is different in all other bacterial sequences analyzed or tested by hybridization. This preferred region of sequence is shown in Figure 1.

20 Because of the extremely large number of organisms potentially relevant to defining exclusivity, and inclusivity (on the order of 7 species and biogroups of Listeria) and the inclusivity characteristics of the test probes, an iterative strategy was adopted to test and refine potential probes. The probes were conveniently synthesized by standard  
25 phosphoramidite (Caruthers, M.H. et al. [1983], in Gene Amplification and Analysis, eds. Papas, T.S., Rosenberg, M., Charikjian, J.G., Pub. Elsevier, New York, Vol. 3 pp.1-26) techniques on an Applied Biosystems instrument.

30 A list of 292 Listeria isolates covering all 7 Listeria species is shown in Table 1. In the preferred assay format, the probes demonstrated excellent inclusivity characteristics - detecting all 292 isolates.

35

A list of non-Listeria bacteria which exemplify the type of bacteria that may be present in potentially Listeria containing samples and which are closely related to Listeria, is given in Table 2. Note that these also represent many of the genera most closely related to Listeria. In the preferred assay format, the probes demonstrated excellent exclusivity characteristics - detecting none of the tested non-Listeria isolates.

A probe which demonstrates good exclusivity characteristics to such a broad representation of bacteria can reasonably be predicted to behave similarly toward a much broader list of more distantly related organisms.

The final steps of probe design and analysis ideally comprise testing real samples (e.g., food/clinical/environmental) and then selecting suitable probes for a final probe set so that the desirable properties are optimized under realistic test conditions.

#### Physical Description of Probes

The foregoing probe selection strategy yielded a number of probes useful for identifying Listeria bacteria in samples. The following preferred oligonucleotide probes are disclosed herein:

Probe 1118: 5'-cTTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGcT-3'

Probe 1212: 5'-cCTCTAACAACAGTACTTTACGATCCGAAAACCTTCTTCATcT-3'

Probe 1120: 5'-CGTCAAGGGACAAGCAGTTACTCTTATCCTTGT-3'

Figure 1 shows these probe sequences (and a number of derivatives thereof) aligned upon their target sequences in the 16S rRNAs of representative Listeria strains. Three groups of probes, corresponding to probes 1118, 1212 and 1120, above, are shown in Figure 1.

Probes of the 1120 group (probes 1120, 1150, 1151 and 1152) are targeted at the region of 16S rRNA sequence corresponding approximately to nucleotide positions 448 to 492 (using the E. coli numbering convention). These probes make use of the region of sequence variability which was discovered to be the most useful for differentiating Listeria from other bacteria, and which is indicated as the region of "core variation" in Figure 1. In the preferred assay format of the present invention (see Example 1) these probes are used to "capture" the target Listeria 16S rRNA molecules in the sample and to remove them from the lysed sample solution. For this purpose, they will have between 20 and 200 deoxyadenosine (dA) residues (not shown in Figure 1) appended to their 3' termini to serve as "handles" for removing the target/probe complex from the sample solution. Under the hybridization stringencies employed in Example 1, probe 1120 is of optimal length and geometry (with respect to the core region of variation) for obtaining the desirable inclusivity and exclusivity behavior characteristics recorded in those experiments. Probes 1150, 1151 and 1152 are longer "versions" of probe 1120 which would be more useful under higher stringency hybridization conditions. Similarly, shorter versions of the 1120 probe, centered around the core region of variation, would be more useful under lower stringency hybridization conditions (e.g., such as those that could be applied in a dot blot format).

The probe group comprised of probes 1212 and 1119 and the "group" represented by probe 1118 are designed as detection probes (according to the previously given definition) and are designed to be used in conjunction with the 1120 series capture probes described above.

Probes 1119 and 1212 are simply length variants of one another. Both are targeted at essentially the same region of Listeria 16S rRNA, spanning nucleotide positions ca. 408 to 447 (using the E. coli numbering convention). In the illustration of the preferred assay format given in Example 1, probe 1119 was utilized. Subsequently, probe 1212 was designed to more closely match the hybridization

characteristics of probe 1120 and probe 1118 with which it is simultaneously used. For purposes of this invention, probes 1119 and 1212 are deemed variations of one another and essentially equivalent in hybridization behavior.

5

The salient features of either probe 1119 and 1212 are: 1) the proximity of their target site to that of probe 1120, and 2) the sequence differences between their target site in Listeria and non-Listeria 16S rRNAs. Although not as strongly different between Listeria and the very closely related Brochothrix as the probe 1120 target sequence, the probe 1119/1212 target sequence nevertheless is variable enough to lend some additional specificity to the probe 1120-containing set. Moreover, the 1119/1212 target sequences diverge to progressively greater extents as one inspects sequences from bacteria more distant from Listeria than Brochothrix (e.g., Bacillus, Staphylococcus, Streptococcus, etc.). The proximity of the probe 1119/1212 target sequence to the target sequence of probe 1120 is important for a number of reasons, including the lessening or abolition of the potential for degradation of the target rRNA by endogenous ribonucleases in the sample. Such degradation would tend to "unlink" the capture and detection probes in the preferred assay format (although other specific features of the preferred assay format also ameliorate this effect) and thereby lessen the observed assay signal. The proximity of the probe 1119/1212 target sequence to the probe 1120 target region also serves to improving the "accessibility" of the probe 1120 target site for hybridization by that probe by disrupting nearby intramolecular structure of the target RNA itself. This tends to generate high overall positive signal levels in the assay.

Like probes 1119 and 1212, probe 1118 also is designed as a detection probe and its target site also flanks the target probe 1120 target site. Probe 1118 adds little if any specificity to the preferred probe set because there is virtually no sequence variation through its target region among the Gram positive bacteria show in Figure 1. Indeed, this region of sequence is nearly constant in all 16S (and eukaryotic 18S)



rRNA so far inspected. The intended functions of probe 1118 are: 1) to promote maximum accessibility of the probe 1120 target site by disrupting native secondary structure in the vicinity of the probe 1120 target site, and 2) to provide incorporation of additional detection  
5 ligand into the captured target/probe complex over and above what can be provided by hybridization of only the 1212 (or 1119) detection probes.

The sequence data in Figure 1 suggest that probes 1118, 1119 and 1120  
10 should hybridize quite widely among Listeria. The five strains of L. monocytogenes (all natural isolates from a variety of food sources) whose 16S rRNAs were inspected in detail all have identical sequences through these target regions (indicated simply as Listeria  
monocytogenes in Figure 1). Likewise, the 16S rRNAs of L. innocua (2  
15 strains), L. seeligeri, L. welshimeri, L. ivanovii, L. grayi and L. murrayi all conform to the L. monocytogenes sequence through these target regions. Some minor variations exist, for example L. grayi and L. murrayi 16S rRNAs also are virtually identical in sequence through  
20 these regions with only one position showing minor variation in the probe 1212/1119 target site (Figure 1, position 434). Nevertheless, this is a small collection of Listeria 16S rRNA sequences compared to the number of known isolates. Our experience with other rRNA-targeted  
Listeria probes (Stackebrandt et al., copending USSN 096,570) and rRNA-  
targeted probes for species of other genera (e.g. Salmonella, USSN  
25 277,579; Yersinia, USSN 169,646; and E. coli, USSN 233,683) have taught us quite clearly that the comparative sequence data can be nothing more than an encouraging first indication that a useful probe might exist. Potentially, much greater sequence variation might exist in other  
Listeria strains not inspected by sequence analysis. Such variation  
30 might reduce or eliminate hybridization by the prospective probes to some or many untested Listeria strains. Therefore, carefully documenting the hybridization behavior of the probes to a large number of Listeria isolates is an important element in documenting that such probes are capable of detecting all Listeria or, failing that, for  
35 documenting which Listeria are not detected. Such hybridization

experiments are described below, the data for which are provided in Table 1. Some 292 Listeria strains, isolated from a wide variety of sources, were tested for reactivity toward the preferred probe set (Example 1), all were strongly positive. This was not a predictable result, even with the fairly extensive 16S rRNA sequence data described above.

Equally as important as the inclusivity behavior of the probes, is their exclusivity, i.e., their reactivity toward non-Listeria bacteria. Particularly important in this regard is their reactivity toward non-Listeria which might also be present in the same environments as Listeria. Results of such exclusivity testing are provided in Table 2. All non-Listeria strains tested (some 27 non-Listeria genera are represented) were negative, i.e. generated absorbancies in the preferred colorimetric assay format of less than 0.1 Absorbance units.

In interpreting these results, it must be borne in the mind that inclusivity and exclusivity behavior are highly interdependent characteristics. To a reasonable approximation both inclusivity and exclusivity behavior of a probe will be a function of: 1) the range of sequence variation found in the probe target regions in target and non-target organisms; 2) the physical characteristics of the probe; and 3) the stringency of hybridization employed. Given any particular target region, one has no experimental control over (1), this is given in nature. As discussed above, (2) and (3) are highly dependent upon one another and, in practice are "adjusted" in parallel. However, in considering any particular probe, one is left only with (3) being available for experimental manipulation. In considering the exclusivity behavior of a particular probe (or probe set) it is therefore important to recognize that inclusivity and exclusivity behavior of a specific probe (or probe set) must be documented under identical or equivalent hybridization conditions.

To understand this interrelationship, consider the following hypothetical example of probe/assay development. A promising target

region is discovered by comparative sequence analysis as discussed above. A test probe is designed which will hybridize to this region. Upon testing under hybridization stringency 1 (HS1), it hybridizes readily to all target organisms (Listeria) tested (an  
5 oversimplification). Upon testing of non-target organisms under (HS1) conditions, some undesirable cross-hybridization of the probe(s) is discovered. To lessen or, more preferably, eliminate this undesirable cross-hybridization, stringency must be increased - e.g. by shortening  
10 the probe, raising the temperature or lowering the salt concentration of the hybridization and/or wash solution. One can systematically or randomly try any number of combinations of any or all these measures - progressively increasing the stringency until the offending cross-hybridization is reduced to "acceptable" levels. Call this  
15 hybridization stringency 2 (HS2). In practice, there is a very real limit on how far stringency of hybridization can be increased. That is, one can only increase stringency to a point where hybridization signal to the target organisms themselves decreases to an  
"unacceptable" level. Such experimental manipulations and limitations are well understood by those skilled in the art of nucleic acid  
20 hybridization.

If the target region in the offending non-target organism is identical to that occurring in one or more of the target organisms then, predictably, no differentiation of target and non-target organisms is  
25 possible with any probes to that target region under any hybridization conditions. This can be determined unambiguously by sequencing the target region of the offending organism.

If the target region in the offending non-target organism is not  
30 identical to that occurring in the target organisms, but only closely related, then it cannot be predicted whether one will be able to discover acceptable stringency conditions (i.e. a given probe or probe set, and a specific set of hybridization conditions) that discriminate target from non-target organisms. In this regard, it shall be noted  
35 that the inclusivity data shown in Table 1 and the exclusivity data

shown in Table 2 were generated with the same probe set (capture probe 1120, detection probes 1118 and 1119) under identical assay conditions. Therefore, under conditions where the assay detects 100% of the Listeria strains tested, it was unexpectedly discovered not to cross-hybridize to any of the tested non-Listeria bacteria.

#### Hybridization Analyses of Probe Behavior

Hybridization behavior of the probes toward representative Listeria and non-Listeria was determined in two basic ways. The first method was the testing under actual assay conditions of pure cultures of numerous Listeria and non-Listeria strains. These data are provided in Tables 1 and 2. This pure culture testing was augmented by hybridization assays performed on cultures of uninoculated and Listeria-containing food and environmental samples. These food and environmental tests ensured that Listeria could be detected under actual "field" conditions in which such a test would be most valuable. They also served to significantly expand the exclusivity testing in a highly relevant manner because cultures from uninoculated samples generally provided high levels of varied non-Listeria bacteria that often are present and selectively enriched in such test samples. To duplicate this range of exclusivity testing using pure cultures of such non-Listeria isolates would be more tedious and potentially irrelevant because the selective enrichment does not necessarily select those non-Listeria organisms most closely related to Listeria. Instead, optimal growth conditions and resistance to antibiotics are the major determinants of isolates which will be present following enrichment. Only a small, representative sampling of the food testing data is shown in Table 3. Overall, some 1500 samples have so far been tested and fully bear out the results indicated in Table 3.

The preferred assay format is described below in Example 1. Example 1 - General, describes the basic assay, including examples of the range of possible variation for some assay steps, which was used to generate the pure culture, liquid hybridization, inclusivity and exclusivity

data shown in Tables 1 and 2. Some of the explicit details of the assay procedure are as given in Example 1 - Specific, which details, in addition, explicit procedures used to inoculate food samples and procedures used for cultivation/enrichment of Listeria from such samples. These data are provided in Table 3.

Example 1 General and Example 1 Specific are descriptions of the same basic assay except in the level of detail provided and in the initial handling of pure culture versus food or environmental samples. All data in Tables 1, 2 and 3 were generated using the probe set 1120 (capture probe), 1118 and 1119 (detection probes) and the same hybridization reagents and conditions.

Example 1 - General: A Homopolymer Capture, Dual Probe, Liquid Hybridization Format

Cultures containing Listeria were grown in modified Listeria enrichment broth (Klinger et al., J. Assoc. Off. Anal. Chem. 71:669-673 [1988]) appropriate for generating selective growth of Listeria. Non-Listeria were grown in non-selective broth, either Tryptic Soy Broth with yeast extract (TSB/Ye, DIFCO) or Brain Heart Infusion Broth (BHI, DIFCO). The nucleic acids then were released by any of a number of appropriate lysis agents (e.g., NaOH, Guanidine salts, detergent, enzymatic treatment, or some combination of the aforementioned). In this example, cells were lysed upon sequential addition of three solution agents: 1) a premixed solution of lysozyme and mutanolysin, 2) proteinase K, and 3) guanidine thiocyanate.

Hybridization was carried out with a set of three probes at least one of which, but not necessarily all three, must be specific for the organism to be detected. In this example, the Listeria specific "capture" probe, probe 1120 was enzymatically tailed with ca.200 deoxyadenosine (dA) residues at its 3' terminus (20-200 dA residues may be used with varying efficiency under different assay conditions). The

reporter probes, 1118 and 1119, could be labeled either chemically or enzymatically with radioactive Phosphorous (P-32) or other small ligand (e.g., fluorescein or biotin) and used to detect the captured target molecules. In this example, the detection probes were labeled with  
5 fluorescein - which was appended chemically to the analog C residues indicated in Figure 1.

Generally, following cultivation/enrichment of bacteria present in the test samples, small aliquots of the cultures were transferred to test  
10 tubes. The bacteria were lysed, the capture and detection probes (diluted in the chemical lysing agent, guanidine thiocyanate) were added. The solution containing the target/probe complex then was brought into contact with a surface containing bound deoxythymidine (dT) homopolymer 1500-4000 nucleotides in length, under conditions that  
15 allowed hybridization between the dA and dT. The hybridization reaction was allowed to proceed for an appropriate period of time (see below, Example 1 - Specific). In this example, the dT was bound to a plastic "dipstick" which was submerged in the target/probe solution. If Listeria ribosomal RNA was present in the test sample, the dA-  
20 tailed, Listeria-specific capture probe (probe 1120) would have hybridized to the target rRNA sequences present and the probe/target complex, in turn, would be captured onto the dipstick. Unhybridized nucleic acids and cellular debris were washed away, leaving the captured DNA-RNA complex attached to the surface via the dA-dT duplex.  
25 The reporter probes also were bound to the dipstick via the chain of interactions - Capture surface-dT:dA-Capture probe:Target:Reporter Probe - only if the correct target nucleic acid was present.

The bound, ligand-derivatized (i.e., fluoresceinated, in this example)  
30 reporter probe then was detected by the addition of the ligand-binding:enzyme complex, anti-fluorescein antibody:horse radish peroxidase, although other alternatives such as streptavidin:alkaline phosphatase, etc. may be employed with appropriately derivatized detection probe.

35

Following incubation under conditions permitting specific formation of the detection complex, washing to remove non-bound enzyme, addition of chromogenic substrate and subsequent color development (typically 20-30 minutes), and the addition of color-termination solution, the developed color was measured spectrophotometrically.

This reading was compared to the negative control levels, a threshold or cutoff value was established, and a determination of the "significance" of the experimental levels was made. Tables 1 and 2 show the results of one such experiment, using pure cultures of various Listeria (Table 1) and non-Listeria (Table 2) bacteria. Overnight cultures of Listeria were diluted 1:100 in Modified Listeria Enrichment Broth immediately prior to the assay. This dilution generally results in a titer of between  $1 \times 10^6$  -  $1 \times 10^7$  colony forming units per milliliter (CFU/ml). All the tested strains were detected with the vast majority of signals falling between 1.0 - 2.0 O.D. 450 units.

As can be seen in Table 2, all non-Listeria tested routinely yield O.D. 450 values of less than 0.1. Thus a positive assay signal is a highly significant indication of the presence of Listeria.

Example 1 - Specific: Detection of Listeria inoculated into food samples.

Some 20 food types have been tested using the preferred probe sets and the preferred assay described herein. Representative experiments with eight food types are presented in Table 3. These food types were selected because they represent a variety of product types that are routinely tested for the presence of Listeria. Uninoculated control samples as well as low and high inocula of four Listeria isolates were tested as follows.

For all food samples, culture enrichment was done by a modification of the FDA reference method (Lovett, J., FDA. Bacteriological Analytical Manual, Suppl.: 529.01-529.12 [9/87]). Twenty-five grams of the food

sample was inoculated into 225 ml of phosphate buffered saline (PBS =  $\text{Na}_2\text{HPO}_4$  1.2 g,  $\text{NaH}_2\text{PO}_4$  0.22 g, NaCl 8.5 g, in a final volume of 1.0 liter of distilled water) and homogenized using a blender or Stomacher. Ten ml of the homogenate was transferred to 225 ml of Modified

5 Enrichment Broth (MEB = Trypticase soy broth 30 g, Yeast extract 6.0 g, 3-[N-Morpholino] propanesulfonic acid - free acid [MOPS-free acid] 8.5 g, 3-[N-Morpholino] propanesulfonic acid - Na salt [MOPS-Na salt] 13.7 g, Acriflavin 15 mg, Nalidixic acid [Na salt] 40 mg, Cycloheximide, 50 mg, in a final volume of 1.0 liter of distilled water) and then

10 incubated in a bottle for 22-26 hours at 35°C.

Secondary enrichment for cultured food samples then was performed. The primary enrichment culture was removed from incubation and mixed well. One ml of the primary enrichment culture was transferred to a bottle

15 containing 100 ml of MEB and incubated for 22-26 hours at 35°C.

For the assay, 0.3 ml of the cultured cells was treated with 0.1 ml of an enzyme solution (150 mg Lysozyme and 3000 units of Mutanolysin in 10 ml of 0.1 M Tris.HCl, 0.01 M EDTA, pH 7.4) and incubated at 37°C for 15

20 minutes, after which 0.1 ml of a lysis solution (50 mg of Proteinase-K in 10 ml of 0.10 M Tris-Buffer, 0.005 M EDTA and 5% Sarkosine pH = 7.5) was added and the incubation was continued at 37°C for an additional 15 minutes. Lysis was complete upon the addition of 0.5 ml of a 65°C

25 solution containing 5.0 M GuSCN, 0.4 M Tris-HCl pH 7.5, 0.08 M EDTA and 0.2mg/ml of dA-tailed 1120 capture probe and 0.3mg/ml each of the fluorescein labeled detector probes 1118 and 1119.

A set of capture dipsticks was placed into the test tubes containing the bacterial lysates and the specific probe set. The tubes were

30 incubated in a 37°C water bath for 60 minutes to enable hybridization of specific capture and reporter probes to target nucleic acids and the capture of these specific DNA/rRNA hybrids to the dipsticks.

After hybridization, the dipsticks were washed by dipping them in a

35 wash basin containing enough wash solution to cover the active part of



the dipstick (2) mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, and 0.1% Tween 20) for 1 minute at room temperature. This process was then immediately repeated in fresh solution at 65°C for one minute. The dipsticks containing the captured target complex were blotted onto  
5 absorbant paper and placed in tubes containing 0.75 ml antibody-enzyme conjugates (anti-fluorescein-horse radish peroxidase diluted in wash buffer), and allowed to incubate at room temperature for 20 minutes.

After allowing the antigen-antibody reaction to occur, the dipsticks  
10 were removed from the test tubes, washed twice in wash buffer at room temperature, blotted and placed into a set of labelled test tubes containing 0.75 ml of substrate-chromogen mixture (2:1 tetramethylbenzidine: hydrogen peroxide, v/v) and allowed to incubate at room temperature for 30 minutes. The dipsticks then were removed  
15 and the color development step terminated by the addition of 0.25 ml 4N sulfuric acid. The optical density (O.D.) of the samples was measured spectrophotometrically at 450nm.

These experimental O.D. values were compared to that obtained using a  
20 non-Listeria, negative control bacterium. In these experiments, Streptococcus faecium was used as a negative control because in earlier, lower-stringency hybridization experiments it was the most difficult to distinguish, non-Listeria bacterium found. S. faecium therefore served as a rigorous control for the proper functioning of  
25 assay components and proper technical execution of the assay. Under the assay conditions described above, S. faecium produced spectrophotometric signals of less than 0.1 O.D. units.

As can be seen in Table 3, there were 51 samples from which Listeria  
30 was isolated from either primary or secondary enrichment broths on selective LPH agar (Lee, W.H., and McClain D., Appl. Environ. Microbiol. 52:1215-1217 [1986]) and also detected by the non-isotopic hybridization assay of the present invention after 48 hour of cultivation.

35

Use of End-Capped Oligonucleotides

5 A further embodiment of this invention incorporates the use of end-capped oligonucleotides. These end-capped probes differ from the uncapped oligonucleotide sequences in that the capped probes contain chemical moieties which are incorporated for the purpose of blocking the 3' and/or 5' terminal hydroxyl groups of the oligonucleotide chain. These blocking groups impart unique stability properties to the probe because they are no longer susceptible to degradation by a variety of  
10 exonucleases which are known to degrade oligonucleotide sequences.

The preparation of the blocked probes can be accomplished using modifications of standard, published methods which are used to synthesize oligonucleotides (S.L. Beaucage and M.H. Caruthers (1981),  
15 Tetrahedron Letters 22, 1859-1862; S. Agrawal, C. Christodoulou and M. Gait (1986), Nucleic Acids Research 14, 6227-6245; J.M. Coull, H.L. Weith and R. Bischoff (1986), Tetrahedron Letters 27, 3991-3994).

20 These modifications incorporate the use of any of a variety of non-nucleoside phosphoramidites which can be attached to the 3' and/or 5' hydroxyl groups of synthetic DNA chains. Since these reagents effectively block one or both of the terminal hydroxyl groups, the resulting synthetic oligonucleotide is resistant to exonuclease digestion.

25 Examples of reagents which can be used in this application include but are not limited to the 5' Amino-modifiers which are available from companies such as Glen Research Corporation (Herndon, VA) or Clontech (Palo Alto, CA). Blocking of the oligonucleotides is accomplished by  
30 adding the non-nucleoside phosphoramidite to the appropriate end or ends of the synthetic oligonucleotide. Generally, the Amino-modifier is dissolved in dry acetonitrile or dichloromethane to a final concentration of 0.1 M. The resulting solution is then placed onto an appropriate port of an automated DNA synthesizer. All of the necessary  
35 operations such as coupling, oxidizing and deblocking of the blocking

reagent are conducted as described in instrument operations manuals such as those which are provided by Applied Biosystems (Foster City, CA) or Biosearch (San Rafael, CA).

5

While the description of the invention has been made with reference to detecting rRNA, it will be readily understood that the probes described herein and probes complementary to those described herein will also be useful to detect the genes (DNA) encoding the rRNA (i.e., rDNA) and accordingly, such probes are to be deemed equivalents to the described probes and encompassed within the spirit and scope of the present invention and the appended claims.

10

TABLE 1  
LISTERIA INCLUSIVITY STUDY

ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. GRAYI	7	CIP6818		1.30	1
L. GRAYI	8	2159		1.56	1
L. INNOCUA	7	CIP8011		1.49	1
L. INNOCUA	10	16-21B-1250		1.96	1
L. INNOCUA	1	33090	6A	1.56	1
L. INNOCUA	8	6719	6A	2.08	1
L. INNOCUA	3	6702	6A	2.15	1
L. INNOCUA	8	6743	6B	1.86	1
L. INNOCUA	8	6766	6B	1.69	1
L. INNOCUA	8	6752	6B	2.26	1
L. INNOCUA	8	6749	6B	0.26	1
L. INNOCUA	8	6738	6B	1.10	1
L. INNOCUA	8	6765	4B	2.27	1
L. INNOCUA	8	6920	6A	2.08	1
L. INNOCUA	8	6541	6A	2.01	1
L. INNOCUA	8	6579	6B	0.82	1
L. INNOCUA	8	6490	6B	2.08	1
L. INNOCUA	8	6880	6B	1.54	1
L. INNOCUA	8	6975	6B	1.72	1
L. INNOCUA	8	6159	6A	2.07	1
L. INNOCUA	8	6881	6B	2.37	1
L. INNOCUA	8	7028	6B	1.62	1
L. INNOCUA	8	7027	6B	1.40	1
L. INNOCUA	8	7113	6B	0.76	1
L. INNOCUA	8	7029	6B	1.96	1
L. INNOCUA	8	7116	6A	0.84	1
L. INNOCUA	8	7028	6B	1.43	1
L. INNOCUA	8	7136	6A	2.26	1
L. INNOCUA	8	7112	6B	0.63	1
L. INNOCUA	3	RM168		1.73	1
L. INNOCUA	3	RM266		1.36	1
L. INNOCUA	3	RM347		1.71	1
L. INNOCUA	3	RM371		2.10	1
L. INNOCUA	3	RM416		1.89	1
L. INNOCUA	2	F8595		1.68	1
L. INNOCUA	2	F8615	6A	2.22	1
L. INNOCUA	2	F2396		2.12	1
L. INNOCUA	2	F8595		2.11	1
L. INNOCUA	1,2	33090, KC1783		2.16	1
L. INNOCUA	4	LA-1		2.20	1
L. IVANOVII	7	CIP7843		1.59	1
L. IVANOVII	8	5756	5	2.26	1
L. IVANOVII	8	6032	5	1.88	1
L. IVANOVII	8	5755	5	1.61	1
L. IVANOVII	8	6736	5	2.10	1
L. IVANOVII	8	3769	5	2.20	1
L. IVANOVII	8	3889	5	1.70	1
L. IVANOVII	8	5378	5	1.62	1
L. IVANOVII	8	5380	5	1.86	1
L. IVANOVII	1,2	19119, KC1786		2.27	1

ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. IVANOVII	2	F6984		2.16	1
L. IVANOVII	4	LA29		2.29	1
L. MONOCYTOGENES	5	3168		1.49	1
L. MONOCYTOGENES	1	15313		1.42	1
L. MONOCYTOGENES	4	000169		1.49	1
L. MONOCYTOGENES	5	3177		1.15	1
L. MONOCYTOGENES	8	Culture A		0.86	1
L. MONOCYTOGENES	8	Culture B		2.09	1
L. MONOCYTOGENES	8	Culture C		1.15	1
L. MONOCYTOGENES	10	170-185-354		0.91	1
L. MONOCYTOGENES	10	141-130-131		1.57	1
L. MONOCYTOGENES	2	15-43B-143		1.16	1
L. MONOCYTOGENES	2	20-C83-P286		1.63	1
L. MONOCYTOGENES	2	19C-84-6807		1.77	1
L. MONOCYTOGENES	7	CIP2754		2.08	1
L. MONOCYTOGENES	7	CIP1929		1.93	1
L. MONOCYTOGENES	7	CIP2750		2.27	1
L. MONOCYTOGENES	7	CIP1890		2.17	1
L. MONOCYTOGENES	7	CIP1826		1.95	1
L. MONOCYTOGENES	7	CIP1826		0.56	1
L. MONOCYTOGENES	7	CIP1817		2.01	1
L. MONOCYTOGENES	7	CIP1820		1.13	1
L. MONOCYTOGENES	7	CIP1820		2.05	1
L. MONOCYTOGENES	1	9644		1.68	1
L. MONOCYTOGENES	1	19111	1	2.14	1
L. MONOCYTOGENES	1	19115	4B	1.39	1
L. MONOCYTOGENES	11	H-1		1.92	1
L. MONOCYTOGENES	11	TYPE 1	1	2.16	1
L. MONOCYTOGENES	11	4131		2.27	1
L. MONOCYTOGENES	11	TYPE-4	4	1.82	1
L. MONOCYTOGENES	11	B64131		2.25	1
L. MONOCYTOGENES	2	F8417		1.07	1
L. MONOCYTOGENES	2	F8833	1/2B	1.30	1
L. MONOCYTOGENES	2	F8935	1/2A	2.11	1
L. MONOCYTOGENES	2	F8921	4B	2.25	1
L. MONOCYTOGENES	2	F7142	4B	1.55	1
L. MONOCYTOGENES	8	7010	4B	1.25	1
L. MONOCYTOGENES	2	F8418	4	2.28	1
L. MONOCYTOGENES	8	6671	4B	1.51	1
L. MONOCYTOGENES	8	5807	3A	1.43	1
L. MONOCYTOGENES	8	6736	1/2B	2.27	1
L. MONOCYTOGENES	8	6655	4B	1.91	1
L. MONOCYTOGENES	8	3559	1/2A	2.05	1
L. MONOCYTOGENES	8	6970	1/2A	2.11	1
L. MONOCYTOGENES	8	6887	4B	1.75	1
L. MONOCYTOGENES	8	6190	1/2A	1.12	1
L. MONOCYTOGENES	8	5877	1/2B	2.17	1
L. MONOCYTOGENES	8	6465	4B	1.08	1
L. MONOCYTOGENES	8	6773	1/2A	1.72	1
L. MONOCYTOGENES	8	8723	1/2B	2.07	1
L. MONOCYTOGENES	8	6797	1/2C	2.13	1
L. MONOCYTOGENES	8	6768	1/2C	0.40	1
L. MONOCYTOGENES	8	6929	1/2B	2.25	1
L. MONOCYTOGENES	8	6763	4B	2.00	1
L. MONOCYTOGENES	8	6483	6B	2.14	1
L. MONOCYTOGENES	8	6721	1/2C	2.16	1
L. MONOCYTOGENES	8	6458	4B	2.14	1

ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. MONOCYTOGENES	8	6787	1/2B	2.26	1
L. MONOCYTOGENES	8	6518	1/2C	1.95	1
L. MONOCYTOGENES	8	6192	3B	2.12	1
L. MONOCYTOGENES	8	6876	1/2A	1.10	1
L. MONOCYTOGENES	8	6762	1/2A	1.90	1
L. MONOCYTOGENES	8	6866	1/2A	2.28	1
L. MONOCYTOGENES	8	6697	1/2A	2.27	1
L. MONOCYTOGENES	8	6537	1/2A	1.83	1
L. MONOCYTOGENES	8	6158	3B	1.01	1
L. MONOCYTOGENES	8	6963	4B	1.86	1
L. MONOCYTOGENES	8	6579	6B	1.81	1
L. MONOCYTOGENES	8	6193	3B	1.28	1
L. MONOCYTOGENES	8	6933	1/2C	1.90	1
L. MONOCYTOGENES	8	5802	3A	2.09	1
L. MONOCYTOGENES	8	6753	3C	2.09	1
L. MONOCYTOGENES	8	6863	1/2A	2.26	1
L. MONOCYTOGENES	8	6560	3B	2.00	1
L. MONOCYTOGENES	8	6869	1/2C	1.90	1
L. MONOCYTOGENES	8	6999	4A	0.83	1
L. MONOCYTOGENES	8	6848	3C	1.90	1
L. MONOCYTOGENES	8	6842	4B	1.26	1
L. MONOCYTOGENES	8	6845	3B	2.14	1
L. MONOCYTOGENES	8	7000	3B	2.13	1
L. MONOCYTOGENES	8	6846	3B	2.24	1
L. MONOCYTOGENES	8	6844	3B	2.26	1
L. MONOCYTOGENES	8	6996	1/2A	1.99	1
L. MONOCYTOGENES	8	6994	1/2A	2.20	1
L. MONOCYTOGENES	8	6990	3A	2.10	1
L. MONOCYTOGENES	8	6840	4C	1.93	1
L. MONOCYTOGENES	8	6838	4B	1.93	1
L. MONOCYTOGENES	8	6995	1/2A	2.27	1
L. MONOCYTOGENES	8	6836	4D	1.51	1
L. MONOCYTOGENES	8	6989	1/2B	2.12	1
L. MONOCYTOGENES	8	6853	3B	1.86	1
L. MONOCYTOGENES	8	3754	4D	2.26	1
L. MONOCYTOGENES	2	F8935	1/2A	1.88	1
L. MONOCYTOGENES	8	7008	1/2A	2.27	1
L. MONOCYTOGENES	8	6988	1/2A	2.28	1
L. MONOCYTOGENES	8	7009	1/2B	1.84	1
L. MONOCYTOGENES	8	6855	4B	2.00	1
L. MONOCYTOGENES	8	6857	1/2C	1.92	1
L. MONOCYTOGENES	8	7010	1/2B	2.03	1
L. MONOCYTOGENES	8	6856	1/2C	2.26	1
L. MONOCYTOGENES	8	7012	4B	1.91	1
L. MONOCYTOGENES	8	7011	1/2B	2.02	1
L. MONOCYTOGENES	8	6854	3B	2.25	1
L. MONOCYTOGENES	8	7007	1/2A	1.93	1
L. MONOCYTOGENES	8	7015	4B	1.95	1
L. MONOCYTOGENES	8	7031A	1/2A	2.13	1
L. MONOCYTOGENES	8	7019	1/2A	2.27	1
L. MONOCYTOGENES	8	6834	4B	1.98	1
L. MONOCYTOGENES	8	7020	1/2A	2.01	1
L. MONOCYTOGENES	8	7083	4A/B	2.07	1
L. MONOCYTOGENES	8	6841	4B	2.07	1
L. MONOCYTOGENES	8	6843	4B	0.98	1
L. MONOCYTOGENES	8	7069	4A/B	1.75	1
L. MONOCYTOGENES	8	7018	1/2B	2.02	1

ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. MONOCYTOGENES	8	7001	4B	1.65	1
L. MONOCYTOGENES	8	47	4A	1.32	1
L. MONOCYTOGENES	8	7065	4A/B	1.40	1
L. MONOCYTOGENES	8	7114	3A	1.87	1
L. MONOCYTOGENES	8	46	4B	1.40	1
L. MONOCYTOGENES	8	49	4A	0.86	1
L. MONOCYTOGENES	8	7032	1/2B	2.15	1
L. MONOCYTOGENES	3	81-861	4B	1.87	1
L. MONOCYTOGENES	2	F2380	4B	2.17	1
L. MONOCYTOGENES	2	F2392	4B	1.61	1
L. MONOCYTOGENES	2	F7288	3A	2.18	1
L. MONOCYTOGENES	1,2	43248, KC1778-H18		2.28	1
L. MONOCYTOGENES	1,2	15313, KC1775		1.98	1
L. MONOCYTOGENES	2	F2381		1.86	1
L. MONOCYTOGENES	2	F8870	4B	2.04	1
L. MONOCYTOGENES	6	592		2.12	1
L. MONOCYTOGENES	6	510		2.19	1
L. MONOCYTOGENES	6	464		2.14	1
L. MONOCYTOGENES	6	474	1/2A	1.91	1
L. MONOCYTOGENES	4	8A5	1	1.99	1
L. MONOCYTOGENES	4	8A34		2.28	1
L. MONOCYTOGENES	4	8A23	1A	2.27	1
L. MONOCYTOGENES	4	SE35	3B	2.30	1
L. MONOCYTOGENES	4	Brie 231	4B	2.17	1
L. MONOCYTOGENES	4	Brie 18	1A	1.78	1
L. MONOCYTOGENES	4	MC145	4B	2.20	1
L. MONOCYTOGENES	4	Scott A	4B	2.09	1
L. MONOCYTOGENES	4	BA-7	4	2.30	1
L. MONOCYTOGENES	4	SF14		2.30	1
L. MONOCYTOGENES	4	DA5	1A	2.06	1
L. MONOCYTOGENES	4	DA-3	4	2.19	1
L. MONOCYTOGENES	4	V7	1A	1.98	1
L. MONOCYTOGENES	9	2662F		1.67	1
L. MONOCYTOGENES	9	Cattle Lavoie		1.81	1
L. MONOCYTOGENES	9	OA-20	4	1.17	1
L. MONOCYTOGENES	9	321LR		1.31	1
L. MONOCYTOGENES	9	266RF		1.48	1
L. MONOCYTOGENES	9	88LR		1.31	1
L. MONOCYTOGENES	9	031852-14		1.92	1
L. MONOCYTOGENES	8	6847	3B	1.91	1
L. MONOCYTOGENES	8	6839	4B	1.86	1
L. MONOCYTOGENES	8	6827	4C	1.48	1
L. MONOCYTOGENES	9	ORF Ferrisburg		1.41	1
L. MONOCYTOGENES	9	Silage-2		1.82	1
L. MONOCYTOGENES	9	675-2634CA	1A	1.77	1
L. MONOCYTOGENES	9	39RF		2.09	1
L. MONOCYTOGENES	9	675-3CA	1A	1.19	1
L. MONOCYTOGENES	9	4841-KO	4	1.73	1
L. MONOCYTOGENES	9	675-14CA	1A	0.46	1
L. MONOCYTOGENES	9	675-14CA	1A	1.96	1
L. MONOCYTOGENES	9	Silage 4		1.85	1
L. MONOCYTOGENES	9	035240-19		2.02	1
L. MONOCYTOGENES	9	Murray B	4B	1.84	1
L. MONOCYTOGENES	9	675-9-16CA	1A	1.61	1
L. MONOCYTOGENES	9	62RR		2.03	1
L. MONOCYTOGENES	9	675-1CA	1A	1.67	1
L. MONOCYTOGENES	9	Bulk Tank		1.22	1

ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. MONOCYTOGENES	9	675-31A		1.96	1
L. MONOCYTOGENES	9	33LF		1.73	1
L. MONOCYTOGENES	9	271RF		1.96	1
L. MONOCYTOGENES	9	50F		2.10	1
L. MONOCYTOGENES	9	Harvester isolate		1.70	1
L. MONOCYTOGENES	9	F5027		2.06	1
L. MONOCYTOGENES	9	675-37CA	1A	1.79	1
L. MONOCYTOGENES	9	75-15CA	4	1.98	1
L. MONOCYTOGENES	9	243D	4	1.33	1
L. MONOCYTOGENES	9	675-16CA	1A	1.94	1
L. MONOCYTOGENES	9	Scott A	4B	2.15	1
L. MONOCYTOGENES	9	675-11CA	1A	2.08	1
L. MONOCYTOGENES	9	29F		2.12	1
L. MONOCYTOGENES	9	675-17CA	1A	2.08	1
L. MONOCYTOGENES	9	675-030CA	1A	2.13	1
L. MONOCYTOGENES	9	OA-1	4	1.85	1
L. MONOCYTOGENES	9	LA16	4	1.74	1
L. MONOCYTOGENES	9	Cow Fetus		1.44	1
L. MONOCYTOGENES	9	F5069	4B	2.11	1
L. MONOCYTOGENES	9	33LR		2.07	1
L. MONOCYTOGENES	9	Mercier	4B	1.91	1
L. MONOCYTOGENES	9	675-17-25CA	1A	2.02	1
L. MONOCYTOGENES	9	675-2CA	1A	1.96	1
L. MONOCYTOGENES	9	31LF		1.39	1
L. MURRAYI	7	CIP76129		0.34	1
L. MURRAYI	1	25401		1.80	1
L. SEELIGERI	7	CIP150100		2.28	1
L. SEELIGERI	2	F7891		1.65	1
L. SEELIGERI	8	3978		1.20	1
L. SEELIGERI	8	6598	6B	1.46	1
L. SEELIGERI	8	6745	4AB	1.39	1
L. SEELIGERI	8	6100	1/2B	2.27	1
L. SEELIGERI	8	6206	6B	2.11	1
L. SEELIGERI	8	6345	1/2A	2.28	1
L. SEELIGERI	8	6101	1/2B	2.19	1
L. SEELIGERI	8	6735	4A	2.28	1
L. SEELIGERI	8	6102	1/2B	2.28	1
L. SEELIGERI	8	4060	1/2B	2.16	1
L. SEELIGERI	8	3663	1/2B	2.28	1
L. SEELIGERI	8	3525	1/2A	2.25	1
L. SEELIGERI	8	3515	1/2A	2.07	1
L. SEELIGERI	8	3479	1/2B	2.20	1
L. SEELIGERI	8	3978	1/2B	1.20	1
L. SEELIGERI	8	6725	4A	2.20	1
L. SEELIGERI	2	KC1785		2.17	1
L. SEELIGERI	2	F7334		2.34	1
L. SEELIGERI	4	Brie 34	1	1.93	1
L. SEELIGERI	4	DA41		2.24	1
L. SEELIGERI	4	MC142		2.15	1
L. SEELIGERI	4	AT2		2.20	1
L. SEELIGERI	4	LA15	1	2.11	1
L. SPP	11	AJ5520		2.03	1
L. SPP	11	3520		2.01	1
L. WELSHIMERI	7	CIP8169		1.39	1
L. WELSHIMERI	2	F8918		1.79	1
L. WELSHIMERI	2,8	5332, KC1825	1/2A	2.26	1
L. WELSHIMERI	8	5828	6A	1.41	1



ORGANISM	SOURCE	I.D. NUMBER	SERO TYPE	O.D. 450nm	HYB+
L. WELSHIMERI	8	5333	6B	1.97	1
L. WELSHIMERI	8	5825	6A	0.92	1
L. WELSHIMERI	8	6199	6A	2.22	1
L. WELSHIMERI	8	5332		2.01	1
L. WELSHIMERI	8	3809	6A	2.25	1
L. WELSHIMERI	3	RM177		2.22	1
L. WELSHIMERI	3	RM213		2.17	1
L. WELSHIMERI	2,8	5333,KC1826	6B	2.22	1
L. WELSHIMERI	2,8	5877,KC1828		2.33	1
L. WELSHIMERI	1,2	ATCC35897,KC1836		2.17	1
L. WELSHIMERI	4	24-34KA	4	0.43	1
L. WELSHIMERI	4	4889A		2.24	1
L. WELSHIMERI	4	2436A	4	1.39	1

## SOURCE KEY:

- 1 AMERICAN TYPE CULTURE COLLECTION  
ROCKVILLE, MD
- 2 CENTERS FOR DISEASE CONTROL  
ATLANTA, GA
- 3 BUREAU OF MICROBIAL HAZARDS  
HEALTH PROTECTION BRANCH  
OTTOWA, CANADA
- 4 U.S. FOOD AND DRUG ADMINISTRATION  
CINCINNAT, OH
- 5 GENE-TRAK SYSTEMS  
FRAMINGHAM, MA
- 6 MASSACHUSETTS DEPARTMENT OF HEALTH  
JAMAICA PLAINS, MA
- 7 PASTEUR INSTITUTE  
PARIS, FRANCE
- 8 SILLIKER LABORATORIES  
CHICAGO HEIGHTS, IL
- 9 UNIVERSITY OF VERMONT  
BURLINGTON, VT
- 10 U.S. DEPARTMENT OF AGRICULTURE  
BELTSVILLE, MD
- 11 YALE UNIVERSITY  
NEW HAVEN, CT

TABLE 2  
 LISTERIA EXCLUSIVITY STUDY

ORGANISM	SOURCE	I.D. NUMBER	O.D. 450 nm	HYB+
ACINETOBACTER CALCOACETIUS	1	ATCC 19606	< 0.1	0
AEROMONAS HYDROPHILIA	1	ATCC 7965	< 0.1	0
BACILLUS BREVIS	1	ATCC 8186	< 0.1	0
BACILLUS CEREUS	1	ATCC 14579	< 0.1	0
BACILLUS COAGULUS	1	ATCC 7050	< 0.1	0
BACILLUS SUBTILIS	1	ATCC 6051	< 0.1	0
BROCHOTHRIX THERMOPHACTA	1	ATCC 11509	< 0.1	0
CITROBACTER DIVERSUS	1	ATCC 27156	< 0.1	0
CITROBACTER FREUNDII	8	S118A	< 0.1	0
CITROBACTER FREUNDII	8	S135	< 0.1	0
CLOSTRIDIUM BIFERMENTANS	1	ATCC 638	< 0.1	0
CLOSTRIDIUM DIFFICILE	1	ATCC 9689	< 0.1	0
CLOSTRIDIUM PERFRINGENS	1	ATCC 3264	< 0.1	0
CLOSTRIDIUM SORDELLII	1	ATCC 9714	< 0.1	0
CORYNEBACTERIUM GENITALIUM	1	ATCC 33031	< 0.1	0
CORYNEBACTERIUM XEROSIS	1	ATCC 373	< 0.1	0
ENTEROBACTER AEROGENES	1	ATCC 13048	< 0.1	0
ENTEROBACTER AGGLOMERANS	8	S121B	< 0.1	0
ENTEROBACTER CLOACAE	8	S135	< 0.1	0
ENTEROCOCCUS HIRAE	1	ATCC 35220	< 0.1	0
ESCHERICHIA COLI	1	ATCC 12036	< 0.1	0
HAFNIA ALVEI	1	ATCC 29927	< 0.1	0
KLEBSIELLA OXYTOCA	1	ATCC 13182	< 0.1	0
KLEBSIELLA PNEUMONIAE	8	S122F	< 0.1	0
KURTHIA ZOPFII	1	ATCC 33403	< 0.1	0
JONESIA DENITRIFICANS	5	GT 0666	< 0.1	0
LACTOBACILLUS ACIDOPHILUS	1	ATCC 4356	< 0.1	0
LACTOBACILLUS MINUTUS	1	ATCC 33267	< 0.1	0
LACTOBACILLUS PLANTARUM	1	ATCC 8014	< 0.1	0
MICROCOCCUS SPECIES	5	GT 0298	< 0.1	0
MICROCOCCUS SPECIES	5	GT 0299	< 0.1	0
MORGANELLA MORGANII	1	ATCC 25830	< 0.1	0
PROTEUS MIRABILIS	5	GT 0364	< 0.1	0
PROTEUS VULGARIS	1	ATCC 29905	< 0.1	0
PROTEUS VULGARIS	8	S133	< 0.1	0
PROVIDENCIA RETTIGERI	1	ATCC 29944	< 0.1	0
PROVIDENCIA STUARTII	1	ATCC 29914	< 0.1	0
PSEUDOMONAS ACIDOVORANS	1	ATCC 15668	< 0.1	0
PSEUDOMONAS AERUGINOSA	5	IG 928	< 0.1	0
PSEUDOMONAS PAUCOMOBILIS	5	GT 0379	< 0.1	0
RHODOCOCCLUS EQUI	1	ATCC 6939	< 0.1	0
SALMONELLA ANATUM	2	715-82	< 0.1	0
SALMONELLA BLEDGAM	2	STK68	< 0.1	0
SALMONELLA TYPHIMURIUM	1	ATCC 23566	< 0.1	0
SERRATIA MARCESENS	1	ATCC 29937	< 0.1	0
SHIGELLA SONNEII	2	STK814	< 0.1	0
STAPHYLOCOCCUS AUREUS	1	ATCC 12600	< 0.1	0
STAPHYLOCOCCUS AUREUS	5	GT 0400	< 0.1	0
STAPHYLOCOCCUS EPIDERMIDIS	5	GT 0402	< 0.1	0

ORGANISM	SOURCE	I.D. NUMBER	O.D. 450 nm	HYB+
STAPHYLOCOCCUS EPIDERMIDIS	5	GT 0403	< 0.1	0
STAPHYLOCOCCUS EPIDERMIDIS	1	ATCC 14990	< 0.1	0
STAPHYLOCOCCUS HOMINIS	1	ATCC 27844	< 0.1	0
STAPHYLOCOCCUS SAPROPHYTICUS	1	ATCC 15305	< 0.1	0
STREPTOCOCCUS AGALACTICAE	1	ATCC 13813	< 0.1	0
STREPTOCOCCUS BOVIS	1	ATCC 15351	< 0.1	0
STREPTOCOCCUS FAECIUM	1	ATCC 6056	< 0.1	0
STREPTOCOCCUS FECALIS	1	ATCC 19433	< 0.1	0
STREPTOCOCCUS GALLINARUM	1	ATCC 35038	< 0.1	0
STREPTOCOCCUS LACTIS	1	ATCC 11454	< 0.1	0
STREPTOCOCCUS MUTANS	1	ATCC 25175	< 0.1	0
STREPTOCOCCUS PNEUMONIAE	1	ATCC 6303	< 0.1	0
STREPTOCOCCUS PYOGENES	1	ATCC 19615	< 0.1	0
STREPTOCOCCUS SALIVARUS	1	ATCC 13419	< 0.1	0
STREPTOCOCCUS SANGUIS	1	ATCC 10556	< 0.1	0
YERSINIA ENTEROCOLITICA	1	ATCC 9610	< 0.1	0

## SOURCE KEY:

- 1 AMERICAN TYPE CULTURE COLLECTION  
ROCKVILLE, MD
- 2 CENTERS FOR DISEASE CONTROL  
ATLANTA, GA
- 5 GENE-TRAK SYSTEMS  
FRAMINGHAM, MA
- 8 SILLIKER LABORATORIES  
CHICAGO HEIGHTS, IL

TABLE 3  
FOOD STUDY

SAMPLE #	STRAIN	CELLS/ SAMPLE	O.D. 450 nm	ASSAY RESULT	MICRO CONFIRM
Total				51	51
1 Raw Shrimp	Listeria monocytogenes	114	1.84	1	1
2 Raw Shrimp	Listeria monocytogenes	11	0.06	0	0
3 Raw Shrimp	control		0.07	0	0
4 Raw Shrimp	Listeria monocytogenes	120	1.39	1	1
5 Raw Shrimp	Listeria monocytogenes	12	0.05	0	0
6 Raw Shrimp	control		0.06	0	0
7 Raw Shrimp	Listeria innocua	76	0.75	1	1
8 Raw Shrimp	Listeria innocua	8	0.04	0	0
9 Raw Shrimp	control		0.04	0	0
10 Raw Shrimp	Listeria ivanovii	58	0.14	1	1
11 Raw Shrimp	Listeria ivanovii	6	0.15	1	1
12 Raw Shrimp	control		0.05	0	0
13 Crabmeat	Listeria monocytogenes	114	1.92	1	1
14 Crabmeat	Listeria monocytogenes	11	0.02	0	0
15 Crabmeat	control		0.04	0	0
16 Crabmeat	Listeria monocytogenes	120	1.88	1	1
17 Crabmeat	Listeria monocytogenes	12	0.05	0	0
18 Crabmeat	control		0.04	0	0
19 Crabmeat	Listeria innocua	76	1.87	1	1
20 Crabmeat	Listeria innocua	8	1.87	1	1
21 Crabmeat	control		0.04	0	0
22 Crabmeat	Listeria ivanovii	58	1.87	1	1
23 Crabmeat	Listeria ivanovii	6	0.04	0	0
24 Crabmeat	control		0.05	0	0
25 Chicken pie	Listeria monocytogenes	114	1.88	1	1
26 Chicken pie	Listeria monocytogenes	11	1.84	1	1
27 Chicken pie	control		0.03	0	0
28 Chicken pie	Listeria monocytogenes	120	1.87	1	1
29 Chicken pie	Listeria monocytogenes	12	1.88	1	1
30 Chicken pie	control		0.03	0	0
31 Chicken pie	Listeria innocua	76	1.87	1	1
32 Chicken pie	Listeria innocua	8	0.00	0	0
33 Chicken pie	control		0.03	0	0
34 Chicken pie	Listeria ivanovii	58	1.77	1	1
35 Chicken pie	Listeria ivanovii	6	1.85	1	1
36 Chicken pie	control		0.03	0	0
37 Beef franks	Listeria monocytogenes	114	1.91	1	1
38 Beef franks	Listeria monocytogenes	11	0.03	0	0
39 Beef franks	control		1.77	1	1
40 Beef franks	Listeria monocytogenes	120	1.88	1	1
41 Beef franks	Listeria monocytogenes	12	0.04	0	0
42 Beef franks	control		0.04	0	0
43 Beef franks	Listeria innocua	76	1.85	1	1
44 Beef franks	Listeria innocua	8	1.90	1	1
45 Beef franks	control		0.02	0	0
46 Beef franks	Listeria ivanovii	58	1.87	1	1
47 Beef franks	Listeria ivanovii	6	1.79	1	1
48 Beef franks	control		0.00	0	0
49 Salami	Listeria monocytogenes	114	1.86	1	1

SAMPLE #	STRAIN	CELLS/ SAMPLE	O.D. 450 nm	ASSAY RESULT	MICRO CONFIRM
50	Salami	Listeria monocytogenes	11	0.00	0
51	Salami	control		0.01	0
52	Salami	Listeria monocytogenes	120	1.74	1
53	Salami	Listeria monocytogenes	12	0.01	0
54	Salami	control		0.12	0
55	Salami	Listeria innocua	76	1.86	1
56	Salami	Listeria innocua	8	1.62	1
57	Salami	control		0.06	0
58	Salami	Listeria ivanovii	58	1.64	1
59	Salami	Listeria ivanovii	6	1.76	1
60	Salami	control		0.05	0
61	Ground turkey	Listeria monocytogenes	114	1.79	1
62	Ground turkey	Listeria monocytogenes	11	1.79	1
63	Ground turkey	control		0.05	0
64	Ground turkey	Listeria monocytogenes	120	1.75	1
65	Ground turkey	Listeria monocytogenes	12	1.69	1
66	Ground turkey	control		0.05	0
67	Ground turkey	Listeria innocua	76	1.49	1
68	Ground turkey	Listeria innocua	8	0.06	0
69	Ground turkey	control		0.03	0
70	Ground turkey	Listeria ivanovii	58	1.73	1
71	Ground turkey	Listeria ivanovii	6	1.71	1
72	Ground turkey	control		1.65	1
73	Surimi	Listeria monocytogenes	114	1.59	1
74	Surimi	Listeria monocytogenes	11	1.68	1
75	Surimi	control		0.04	0
76	Surimi	Listeria monocytogenes	120	1.73	1
77	Surimi	Listeria monocytogenes	12	1.71	1
78	Surimi	control		0.05	0
79	Surimi	Listeria innocua	76	1.64	1
80	Surimi	Listeria innocua	8	1.61	1
81	Surimi	control		0.06	0
82	Surimi	Listeria ivanovii	58	1.64	1
83	Surimi	Listeria ivanovii	6	0.03	0
84	Surimi	control		0.03	0
85	Cabbage	Listeria monocytogenes	114	0.99	1
86	Cabbage	Listeria monocytogenes	11	0.05	0
87	Cabbage	control		0.05	0
88	Cabbage	Listeria monocytogenes	120	1.77	1
89	Cabbage	Listeria monocytogenes	12	0.04	0
90	Cabbage	control		0.06	0
91	Cabbage	Listeria innocua	76	1.74	1
92	Cabbage	Listeria innocua	8	1.66	1
93	Cabbage	control		0.03	0
94	Cabbage	Listeria ivanovii	58	1.63	1
95	Cabbage	Listeria ivanovii	6	1.44	1
96	Cabbage	control		0.03	0

=====

Assay Cut off = 0.12

What is claimed is:

1. A nucleic acid fragment stablized against exonucleases degradation and capable of hybridizing, under predetermined stringency conditions, to rRNA or rRNA gene (rDNA) of Listeria and not to rRNA or DNA of non-Listeria.  
5
2. The nucleic acid fragment of Claim 1 wherein said fragment is not capable of hybridizing, under said conditions, to rRNA or rDNA of Acinetobacter calcoacetius, Aeromonas hydrophilia, Bacillus brevis, Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Brochothrix thermosphacta, Citrobacter diversus, Citrobacter freundii, Clostridium bifermentans, Clostridium difficile, Clostridium perfringens, Clostridium sordellii, Corynebacterium genitalium, Corynebacterium xerosis, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Enterococcus hirae, Escherichia coli, Hafnia alvei, Klebsiella oxytoca, Klebsiella pneumoniae, Kurthia zopfii, Jonesia denitrificans, Lactobacillus acidophilus, Lactobacillus minutus, Lactobacillus plantarum, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia rettigeri, Providencia stuartii, Pseudomonas acidovorans, Pseudomonas aeruginosa, Pseudomonas paucomobilis, Rhodococcus equi, Salmonella anatum, Salmonella bledgum, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus saprophyticus, Streptococcus agalacticae, Streptococcus bovis, Streptococcus faecium, Streptococcus faecalis, Streptococcus gallinarum, Streptococcus lactis, Streptococcus mutants, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus sanguis and Yersinia enterocolitica.  
10  
15  
20  
25  
30
3. The nucleic acid fragment of Claim 1, comprising a probe sequence selected from the group of probes consisting of probe 1120, 1150, 1151 and 1152.  
35

4. The nucleic acid fragment of Claim 1, comprising a sequence selected from the group of sequences complementary to probes 1120, 1150, 1151 and 1152.
5. The nucleic acid fragment of Claim 1 wherein said fragment is complementary to at least 95% of a sequence comprising any ten consecutive nucleotides within region 452 to 484 of the 16S rRNA sequence of Listeria monocytogenes shown in Figure 1.
- 10 6. The nucleic acid fragment of Claim 1 wherein said fragment is homologous to at least 95% of a sequence comprising any ten consecutive nucleotides within region 452 to 484 of the 16S rRNA sequence of Listeria monocytogenes shown in Figure 1.
- 15 7. A set of probes comprising at least two nucleic acid fragments, one of which is the probe of Claim 3 and the other of which is selected from the group of probes consisting of 1119, 1212 and 1118.
- 20 8. A set of probes comprising at least two nucleic acid fragments, one of which is the probe of Claim 4 and the other of which is selected from the group of probes consisting of sequences complementary to probes 1119, 1212 and 1118.
- 25 9. The set of probes of Claim 7 comprising i) probe 1120 and probe 1119, and ii) a sequence complementary to each of probe 1120 and probe 1119.
- 30 10. The set of probes of Claim 7 comprising i) probe 1120 and probe 1212, and ii) a sequence complementary to each of probe 1120 and probe 1212.

11. The set of probes of Claim 7 comprising i) probe 1120 and probe 1118, and ii) a sequence complementary to each of probe 1120 and probe 1212.
- 5 12. A set of probes comprising i) probe 1212 and probe 1118, and ii) a sequence complementary to each of probe 1212 and probe 1118.
13. A method for detecting the presence of Listeria in a sample comprising:
- 10 a) contacting said sample with at least one nucleic acid fragment stabilized against exonucleases activity and selected from the group consisting of 1120, 1150, 1151, 1152, 1119, 1212, 1118 and a sequence complementary to any of the foregoing, under conditions that allow said fragment to hybridize to rRNA or rDNA genes (rDNA) of Listeria, if present in said sample, to form hybrid nucleic acid complexes, wherein said nucleic acid fragment is capable of hybridizing, under predetermined stringency conditions, to rRNA or rDNA of Listeria and not to rRNA or rDNA of non-Listeria; and
- 15 20 b) detecting said hybrid nucleic acid complexes as an indication of the presence of said Listeria in said sample.
- 25 14. The method of Claim 13 wherein said nucleic acid fragment of said contacting step is selected from the group consisting of 1120, 1150, 1151 and 1152.
- 30 15. The method of Claim 13 wherein said nucleic acid fragment of said contacting step is selected from the group consisting of a sequence complementary to a probe selected from the group of 1120, 1150, 1151 and 1152.
- 35



16. The method of Claim 13 wherein contacting step comprises contacting said sample with at least two nucleic acid fragments, one of which is selected from the group consisting of 1119, 1212, 1118 and sequences complementary thereto, and one of which is selected from the group consisting of 1120, 1150, 1151, 1152 and sequences complementary thereto.
17. The method of Claim 13 wherein said contacting step comprises contacting said sample with at least two nucleic acid fragments, one of which is selected from the group consisting of a sequence complementary to each of 1119, 1212 and 1118, and one of which is selected from the group consisting of a sequence complementary to each of 1120, 1150, 1151 and 1152.
18. The method of Claim 16 wherein said contacting step comprises contacting said sample with nucleic acid fragments 1120 and 1119.
19. The method of Claim 16 wherein said contacting step comprises contacting said sample with nucleic acid fragments 1120 and 1212.
20. The method of Claim 16 wherein said contacting step comprises contacting said sample with nucleic acid fragments 1120 and 1118.
21. The method of Claim 16 wherein said contacting step comprises contacting said sample with nucleic acid fragments selected from the group of sequences complementary to each of 1212 and 1118, and sequences 1212 and 1118.
22. A method for detecting the presence of Listeria in a sample comprising:

- a) contacting at least one nucleic acid fragment, stabilized against exonucleases activity, with said sample under conditions that allow said fragment to hybridize to rRNA or rRNA genes (rDNA) of Listeria, if present in said sample, to form hybrid nucleic acid complexes, wherein said nucleic acid fragment is capable of hybridizing to rRNA or rDNA of Listeria and not to rRNA or rDNA of Acinetobacter calcoaceticus, Aeromonas hydrophilia, Bacillus brevis, Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Brochothrix thermosphacta, Citrobacter diversus, Citrobacter freundii, Clostridium bifermentans, Clostridium difficile, Clostridium perfringens, Clostridium sordellii, Corynebacterium genitalium, Corynebacterium xerosis, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Enterococcus hirae, Escherichia coli, Hafnia alvei, Klebsiella oxytoca, Klebsiella pneumoniae, Kurthia zopfii, Jonesia denitrificans, Lactobacillus acidophilus, Lactobacillus minutus, Lactobacillus plantarum, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia rettigeri, Providencia stuartii, Pseudomonas acidovorans, Pseudomonas aeruginosa, Pseudomonas paucomobilis, Rhodococcus equi, Salmonella anatum, Salmonella bledgum, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus saprophyticus, Streptococcus agalacticae, Streptococcus bovis, Streptococcus faecium, Streptococcus faecalis, Streptococcus gallinarum, Streptococcus lactis, Streptococcus mutants, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus sanguis and Yersinia enterocolitica; and

- b) detecting said hybrid nucleic acid complexes as an indication of the presence of said Listeria in said sample.

5

23. The method of Claim 22 wherein said nucleic acid fragment is complementary to at least 95% of a sequence comprising any ten consecutive nucleotides within region 452 to 484 of the 16S rRNA sequence of Listeria monocytogenes shown in Figure 1.

10

24. The method of Claim 22 wherein said nucleic acid fragment is homologous to at least 95% of a sequence comprising any ten consecutive nucleotides within region 452 to 484 of the 16S rRNA sequence of Listeria monocytogenes shown in Figure 1.

15

25. An assay kit for detecting Listeria comprising at least one nucleic acid fragment of Claim 2 packaged in at least one container, and instructions for utilizing at least one nucleic acid fragment for detecting Listeria.

20

26. An assay kit for detecting Listeria comprising the nucleic acid fragments of Claim 7 packaged in at least one container, and instructions for utilizing said nucleic acid fragments for detecting Listeria.

25

FIGURE 1: PROBES AND RIBOSOMAL RNA TARGET SEQUENCES

E. coli position #	408	447
<i>Escherichia coli</i>	5'....GUAUGAAGAAGGCCUUCGGGUUGUAAAGUACUUUCAGCGGGG	
Probe 1119		TcCTTCCAAAAGCCTAGCATTTTCATGACAACAATCTCC-5'
Probe 1212		TcTACITCTTCCAAAAGCCTAGCATTTTCATGACAACAATCTCC-5'
<i>Listeria monocytogenes</i>		...GUAUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria innocua</i>		...GUAUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria seeligeri</i>		...GUAUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria welshimeri</i>		...GUAUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria ivanovii</i>		...GUAUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria grayi</i>		...GUGUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Listeria murrayi</i>		...GUGUGAAGAAGGUUUUUCGGAUCGUAAGUACUGUUUAGAG
<i>Brochothrix thermosphacta</i>		...GAGCGAAGAAGGCCUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Bacillus subtilis</i>		...GAGUGAUGAAGGUUUUUCGGAUCGUAAGCUCUGUUUAGAG
<i>Bacillus cereus</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Staphylococcus aureus</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Staphylococcus epidermidis</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Enterococcus faecalis</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Clostridium innocuum</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Mycobacterium capricolum</i>		...GAGUGAUGAAGGUUUUUCGGGUCGUAAGCUCUGUUUAGAG
<i>Arthrobacter globiformis</i>		...GAGGGAUGACGGCCUUCGGGUGUAAACCUCUUUCAGUAGGG
<i>Streptomyces violaceoruber</i>		...GAGGGAUGACGGCCUUCGGGUGUAAACCUCUUUCAGCAGGG

E. coli position # (cont'd)	448	452	456	477	484	492
<i>Escherichia coli</i>						
	AGGAAGG-	GAGUAAAGUUAUACCUUGCUCAUUGACGUUACCGC				
Probe 1152	TTCTTGTTCC	TATTCATTGACGAA-CAGGGA	ACTGCCATAGATT-5'			
Probe 1151	TTCTTGTTCC	TATTCATTGACGAA-CAGGGA	ACTGCCAT-5'			
Probe 1150	TGTTCC	TATTCATTGACGAA-CAGGGA	ACTGCCAT-5'			
Probe 1120	TGTTCC	TATTCATTGAC 3AA-CAGGGA	ACTGTC-5'			
Core Variation	*****					
<i>Listeria monocytogenes</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Listeria innocua</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Listeria seeligeri</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Listeria welshimeri</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Listeria ivanovii</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Listeria grayi</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUA...					
<i>Listeria murrayi</i>	AAGAACAAAGGAUAAGAGUAACUGCUU-GUCCCUUGACGGUAUCUAA					
<i>Brochothrix thermosphacta</i>	AAGAACAAAGGUGAGAGUAACUGUUC-ACCCUUGACGGUAUCUAA					
<i>Bacillus subtilis</i>	AAGAACAAAGUACCGUUCGACAGGCGGUACCUUGACGGUACCUAA					
<i>Bacillus cereus</i>	AACAACAAGUGCUAGUUAAGUAGCUGGCACCUUGACGGUACCUGA					
<i>Staphylococcus aureus</i>	AAGGACAAAGGUGUNAGUAACUGUGC-ACAUCUUGACGGUACCUGA					
<i>Staphylococcus epidermidis</i>	AAGAACAAAGGUGUAAGUAACUGUGC-ACGUCUUGACGGUACCUAA					
<i>Enterococcus faecalis</i>	AAGAACAAAGGACGUUAGUAACUGAAC-GUCCCUUGACGGUACCUAA					
<i>Clostridium innocuum</i>	AAGAACGGCUCUAAGAGGAAUUGCUA-UGGAGUGACGGUACCUUA					
<i>Mycobacterium capricolum</i>	AACAATAAUAAGUAGGAAUAGACU-UUAUCUUGACAGUACCUUA					
<i>Arthrobacter globiformis</i>	AAGAAGCG-----					
<i>Streptomyces violaceoruber</i>	AAGAAGCG-----					

E. coli position # (cont'd) 493	533
Escherichia coli	AGAAGAAGCACCGGCUAACUCGUGGCCAGCAGCCGGCGGUAUAUACGG...3'
Probe 1118	TcGTCTTTTCGGTCCCGATTGATGCACGGTCTCGGGCCCATTC-5'
Listeria monocytogenes	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Listeria innocua	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Listeria seeligeri	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Listeria welshimeri	CCAGAAAGCnnCGGCUAACUACGUGCCAGCAGCnCGGCGUAUAUACGU...
Listeria murrayi	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCCGGCGUAUAUACGU...
Brochothrix thermosphacta	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Bacillus subtilis	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Bacillus cereus	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Staphylococcus aureus	UCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Staphylococcus epidermidis	UCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Enterococcus faecalis	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Clostridium innocuum	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Mycobacterium capricolum	CCAGAAAGCCACGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Arthrobacter globiformis	AGAAGAAGCCCGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...
Streptomyces violaceoruber	AGAAGAAGCCCGGCUAACUACGUGCCAGCAGCCGGCGGUAUAUACGU...

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/00656

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : C 12 Q 1/68, C 07 H 21/04		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	C 12 Q	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> *		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
P, X	EP, A, 0314294 (GENE-TRAK SYSTEMS) 3 May 1989 see the whole document, especially column 3, lines 40-54; columns 5-8	1, 2, 22
Y	Proc. Natl. Acad. Sci. USA, vol. 84, August 1987, N.T. Thuong et al.: "Oligo(alpha- deoxynucleotide)s covalently linked to intercalating agents: differential binding to ribo- and deoxyribopoly- nucleotides and stability towards nuclease digestion", pages 5129-5133, see "Introduction"	1, 2
A	GB, A, 2169403 (ORION YHTYMA OY) 9 July 1986	13, 22
<p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
7th June 1990	02.07.90	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	H. Daniels H. DANIELS	

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see the whole document --	
P,A	WO, A, 89/06699 (INSTITUT PASTEUR) 27 July 1989 see abstract; claims -----	22

Form PCT ISA 210 (extra sheet) (January 1985)



# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9000656  
SA 35099

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/06/90. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0314294	03-05-89	AU-A- 2213488	16-03-89
GB-A- 2169403	09-07-86	AU-B- 561382	07-05-87
		AU-A- 5174885	17-07-86
		BE-A- 903937	16-04-86
		CH-A- 666696	15-08-88
		DE-A- 3546312	10-07-86
		FR-A- 2575493	04-07-86
		JP-A- 61185200	18-08-86
		LU-A- 86238	14-04-86
		NL-A- 8503597	01-08-86
		SE-A- 8600011	03-07-86
WO-A- 8906699	27-07-89	EP-A- 0355147	28-02-90

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82